Determination of Bioactive Components of Decholestrate, a polyherbal formulation by GC-MS Analysis

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Abstract: In this study, the bioactive compounds of Decholestrate, a polyherbal formulation, have been evaluated using GC-MS technique. The chemical composition of the aqueous extract of decholestrate was investigated using Perkin-Elmer Gas Chromatography - Mass Spectroscopy. This analysis revealed the presence of Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- (23.79), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- [S-(Z)]- (13.80), 1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one (13.05). The compounds found in the study are reported to possess cardio toning properties and anti-hyperlipidemic activity. The result of this study offers a platform to reconfirm the properties of the components in decholestrate that are used as antihyperlipidemic agents.
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1. Introduction

Plants serve as a basis of traditional medicinal systems for thousands of years (Hammer, 1999). Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant (Koduru *et al.,* 2006). Plants produce a remarkable diverse array of over 5,00,000 low and high molecular mass natural products which are known as secondary metabolites (Fatope, 2001). Distinguished example of these compounds includes flavonoids, phenols, saponins and cyanogenic glycosides (Shahidi, 2000 and Shahidi *et al*., 2008). It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998).

Decholestrate is a polyherbal formulation that comprises of the extracts of the plant constituents from *Zingiber officinale* (Ginger), *Tinospora cordifolia* (Guduchi), *Piper longum* (long pepper), *Phyllanthus emblica* (Amla), *Embellia ribes* (False black pepper), *Vigna unguiculata* (Cow pea), *Garcinia cambogia* (Gambooge), *Commiphora mukul* (Guggul), *Camellia sinensis* (Tea leaves). The above herbs are reported to possess anti-diabetic, antihyperlipidemic (Al-Amin *et al.,* 2006), antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic and anti-diabetic (Singh *et al.,* 2003), antimicrobial (Ali *et al.,* 2007), antioxidant (Siddhuraju and Becker 2007), antiulcerogenic, hepatoprotective (Krishnaveni and Mirunalini, 2010), antibacterial (Radhakrishnan *et al.,* 2011), anti-obesity (Heymsfield *et al.,* 1998), hypolipidemic (Wang *et al.,* 2004) and anti-carcinogenic properties (Hamilton-Miller, 2001) respectively. The aim of this study is to determine the organic compounds present in this formulation with the aid of GC-MS technique, which may provide an insight in its use as an antihyperlipidemic agent.

2. Materials and Methods

Plant material

Fresh plant/plant parts were purchased from local market in Coimbatore, Tamilnadu and Thrissur, Kerala, India. The taxonomic identities of these plants were confirmed and the voucher specimen numbers of the plants were deposited at Phytopharma testing lab, T. Stanes Company Ltd. Coimbatore. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Plant extraction procedure

10 g of air-dried powder of the plant constituents was added to distilled water [1:10] and boiled on slow heat for 2 h with periodical stirring. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000rpm for 10 mins. The supernatant was collected and the above procedure was repeated thrice. After 6 hours, the supernatant collected at an interval of every 2 hours were pooled together and concentrated to make the final volume one-fourth of the original volume (Harbone, 1973). It was then

autoclaved at 121° C and at 15 lbs pressure and stored at 4° C. The resulted powder was collected for every individual plant. These powders in equal proportions were mixed and homogenised in distilled water [1:20] at 60 ° C to form a concoction. It was then vaccum dried and used for the GCMS analysis.

Gas Chromatography – mass spectrum analysis (GC-MS)

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions. Column Elite-1 fused silica capillary column (30mm x 0.25mm ID x 1 µ Mdf, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; Helium (99.999%) was used as carrier gas at a constant flow of 1ml /min and an injection volume of 2 ml was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. the oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C / min, to 200°C then 5°C / min, to 280°C, ending with a 9 min Isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage of each component was calculated by comparing its average peak area to the total area. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver5.2.0.

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, structure of the components in the test material was ascertained.

3. Results

Fourteen compounds were identified in Decholestrate formulation by GC-MS analysis. The active principle, Molecular weight (MW), Concentration (%), Molecular formula (MF), Retention Time (RT) and their bioactivity are presented in Figure 1, Table 1 and 2 dictates that the predominant compounds are Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl- (23.79), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]- (13.80), 1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one (13.05), and Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]- (11.26).

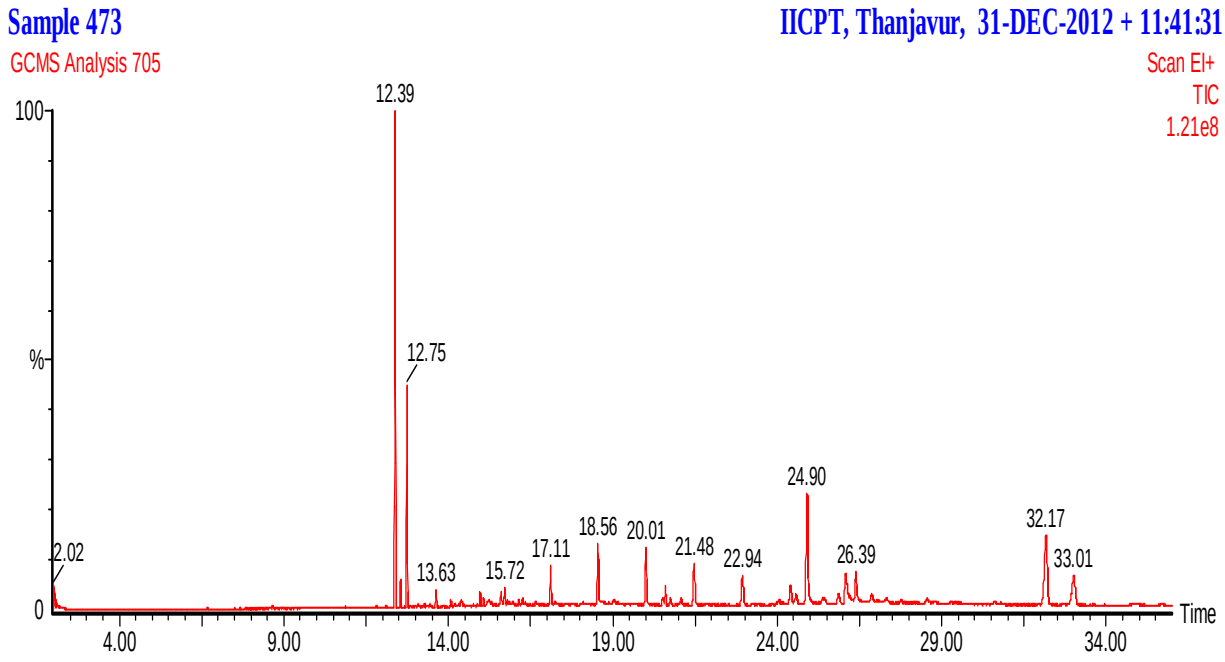

Figure 1. Chromatogram obtained from the GC-MS with the extract of Decholestrate

Table 1. Total ionic chromatogram (GC-MS) of decholestrate obtained with 70eV using an Elite -1 fused silica capillary column with He gas as the carrier.

No.	RT	Name of the compound	Molecular formula	MW	Peak Area%
1	12.39	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	$C_{15}H_{24}$	258	23.79
2	12.75	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	$C_{15}H_{24}$	204	11.26
3	13.63	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	$C_{15}H_{26}O$	222	1.12
4	15.72	1-Iodo-2-methylnonane	$C_{10}H_{21}I$	268	1.04
5	17.11	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	2.83
6	18.56	Eicosane	$C_{20}H_{42}$	282	5.22
7	20.01	Tridecane, 3-methyl-	$C_{14}H_{30}$	198	5.22
8	21.48	Heptadecane, 2,6-dimethyl-	$C_{19}H_{40}$	268	4.47
9	22.94	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_{30}$	198	3.50
10	24.90	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	$C_{18}H_{26}O$	258	13.05
11	26.09	Piperine	$C_{17}H_{19}NO_3$	285	4.92
12	26.39	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	$C_{15}H_{26}$	206	3.28
13	32.17	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	$C_{15}H_{26}O$	222	13.80
14	33.01	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	6.49

Table 2. Activity of the components identified in Decholestrate [GC MS study]

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound Nature	**Activity
1	12.39	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	$C_{15}H_{24}$	258	23.79	Sesquiterpene compound	Antimicrobial, Anti-inflammatory, Anti hyperlipidemic
2	12.75	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	$C_{15}H_{24}$	204	11.26	Sesquiterpene compound	Antimicrobial, Anti-inflammatory, Anti hyperlipidemic
3	13.63	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	$C_{15}H_{26}O$	222	1.12	Sesquiterpene alcohol	Antimicrobial, Anti-inflammatory, Anti hyperlipidemic
4	15.72	1-Iodo-2-methylnonane	$C_{10}H_{21}I$	268	1.04	Iodo compound	Antimicrobial
5	17.11	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	2.83	Iodo compound	Antimicrobial
6	18.56	Eicosane	$C_{20}H_{42}$	282	5.22	Alkane	No activity reported
7	20.01	Tridecane, 3-methyl-	$C_{14}H_{30}$	198	5.22	Alkane compound	No activity reported
8	21.48	Heptadecane, 2,6-dimethyl-	$C_{19}H_{40}$	268	4.47	Alkane compound	No activity reported
9	22.94	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_{30}$	198	3.50	Alkane compound	No activity reported
10	24.90	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	$C_{18}H_{26}O$	258	13.05	Ketone compound	No activity reported

11	26.09	Piperine	$C_{17}H_{19}NO_3$	285	4.92	Alkaloid	ATPase-Stimulant Adrenergic Analgesic Anesthetic Antiaflatoxin Antibacterial; Anticlastogen Anticonvulsant Antiedemic Antifertility Antiimplantation Antiinflammatory Antileishmanic Antimutagenic Antinarcotic; Antioxidant Antiplasmodial Antipyretic; Antiseptic Antispasmodic Aryl-Hydrocarbon-Hydroxylase-Inhibitor CNS-Stimulant Cardio tonic Carminative; Catecholaminogenic Diaphoretic Endorphinogenic Epinephrininergic FLavor; Hepatoprotective Insecticide Parasiticide Pesticide; Respirostimulant Secretogogue Serotoninergic
12	26.39	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-	$C_{15}H_{26}$	206	3.28	Sesquiterpene compound	Antimicrobial, Anti-inflammatory, Anti hyperlipidemic
13	32.17	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	$C_{15}H_{26}O$	222	13.80	Sesquiterpene alcohol	Antimicrobial, Anti-inflammatory, Anti hyperlipidemic
14	33.01	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}O$	222	6.49	Sesquiterpene alcohol	Antimicrobial, Anti-inflammatory, Anti hyperlipidemic

****Activity source**: Dr Duke's Phytochemical and Ethnobotanical data base.

4. Discussions

The results obtained show that majority of the predominant compounds in the formulation are found to be sesquiterpene compounds. Sesquiterpenes were reported to have anti-hyperlipidemic activity. The oxygen functional groups at the 3- and 8-positions and exo-methylene moiety in alpha-methylene-gamma-butyrolactone ring were found to be essential for the anti-hyperlipidemic activity of guaiane-type sesquiterpene (Shimoda *et al.,* 2003). Piperine, another component in the formulation, is a potent cardio toner. Thus based on these results it may be concluded that the components of decholestrate possess anti-hyperlipidemic activity. Further *in vivo* studies may be carried out to reconfirm the lipid lowering activity of the formulation.

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